# Metabolism of Natural Retinoids in Psoriatic Epidermis

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Synthetic retinoids that have distinct therapeutic activity on psoriatic plaques act at least in part through the metabolic pathways of natural retinoids. The metabolism of retinol, retinal, and retinoic acid in human epidermis in general and psoriatic plaques in particular has not been previously analyzed. This appears to be an important issue, because enzymatic formation of retinoic acid from retinol within target cells is thought to be the source of biologically active retinoic acid therein and might be subjected to regulation. We found that the enzymatic system that transforms ($^3$H)retinol into ($^3$H)retinoic acid is detectable in psoriatic plaques ($0.33 \pm 0.07$ pmol/h/mg protein) but only in trace amounts in normal epidermis. The activity due to alcohol deshydrogenase seems not to be involved in this process. ($^3$H)retinal was found to be either reduced into retinol or oxidized into retinoic acid, depending on the ratio of NAD to NADH (the oxidized and reduced nicotinamide-adenine dinucleotide, respectively); the former reaction was higher in psoriatic plaques than in normal epidermis. *J Invest Dermatol 95:47S–48S, 1990*

Synthetic retinoids are therapeutically active compounds used in the treatment of psoriasis. It is most likely that these drugs act, in part, through the pathway of natural retinoids. Therefore, the metabolism and transport of natural retinoids (i.e., retinol, retinal, and retinoic acid) are worth studying in psoriatic epidermis. Not all aspects of this metabolism are presently accessible to analysis, however. Of the key steps in this metabolism (Table I) only a few have already been considered.

We have previously found an important increase of cellular retinoic acid-binding protein (CRABP) in lesional psoriatic epidermis as compared with non-involved epidermis [1], whereas CRBP did not change. Although CRABP is not essential for the effects of RA in some cell lines, where direct access of RA to the nuclear receptor (RAR) appears to happen [2], an important physiologic function for CRABP in vivo is likely. The significance of the increase in CRABP in psoriatic lesional epidermis has not been established, but indirect observations have suggested that it could be related to an increase either in ligand loading or ligand need [1,3,4]. The origin of the RA used by a given cell in vivo is still debated; serum levels of RA are about 20 nM [5], which is approximately 150 times less than the levels of retinol. Current thought favors the enzymatic formation of RA from retinol within the target cells as a source of biologically active RA [6]. If so, the enzymatic system that generates RA should be considered as a potentially important regulatory step up-stream to the nuclear receptor.

Enzymatic conversion of retinol into RA has been previously identified in pig kidney cell lines [7] and rat tissues [8], as well as in mouse epidermis [9]. The concentrations of retinol used in this latter study (up to 1 mM) were not physiologic, and a non-specific alcohol deshydrogenase, rather than retinol deshydrogenase, was found to be involved [9]. It was, therefore, necessary to use more sensitive techniques for the detection of retinol metabolites at the femtomolar level per assay, thereby allowing the study of physiologic levels of retinol.

Recently we have studied the metabolism of retinol and retinal by keratinocytes by using two approaches: i) the analysis by HPLC of the metabolites produced when cytosolic extracts of human keratinocytes are incubated with either 600 nM of ($^3$H)retinol or 600 nM of ($^3$H)retinal, and ii) the formation of a CRABP peak after incubation with ($^3$H)retinol (this "indirect" approach not only shows the generation of RA from retinol but also that the RA produced binds to CRABP). These techniques were first applied to the cytosolic extracts of normal human epidermal slices (keratome set at 180 $\mu$m), where no measurable enzymatic activities were found. We hypothesized that the conversion of retinol into RA might not occur at a similar rate during the process of epidermal differentiation; if so, the levels of the enzyme(s) responsible may be too low in epidermal extracts made from keratinocytes at different stages of differentiation. Therefore, we used cultured human keratinocytes in which differentiation was modulated by extracellular calcium concentration [3]. The cytosols of differentiating keratinocytes were found to generate ($^3$H)RA at a rate of $4.49 \pm 0.17$ pmol/h/mg protein when exposed to ($^3$H)retinol; and the RA formed did bind to CRABP as shown by a PAGE radio-binding assay [10,11]. The enzymatic activity differed from the alcohol metabolizing enzymes (alcohol and aldehyde deshydrogenases) because the formation of RA was not affected by the specific alcohol metabolism inhibitors 4 methyl pyrazole and disulfiram. In contrast, the cytosol of non-differentiated keratinocytes, despite the presence of alcohol deshydrogenase activity in the same amounts as in differentiating keratinocytes, did not generate RA from retinol at a measurable level. This was related to the absence of retinol deshydrogenase activity in non-differentiated keratinocytes; when ($^3$H)retinal was used as substrate, ($^3$H)RA was formed. In fact, the two cell populations were found to reduce retinal into retinol at a similar rate (8 pmol/h/mg protein) in the presence of the appropriate co-factor (NADH). On the other hand, ($^3$H)RAL was oxidized to RA in the presence of NAD at a higher rate in human differentiating keratinocytes (51.6 pmol/h/mg) as compared to non-differentiated keratinocytes (14.4 pmol/h/mg).

This investigation was supported in part by the Swiss National Science Foundation, grant no. 3.874.0.88.

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Abbreviations:
CRABP: cellular retinoic acid-binding protein
CRBP: cellular retinol-binding protein
NAD: nicotinamide-adenine dinucleotide, oxidized form
NADH: reduced NAD
RA: retinoic acid
RAL: retinal
ROL: retinol

0022-202X/90/$03.50 

**Table I.** Key Points for the Demonstration of a Defect in the Physiology of Natural Retinoids in Psoriasis

1. Cell membrane receptor (RBP)
2. Intracellular retinol
3. CRBP
4. Enzymatic conversion of ROL into RA
5. CRABP
6. Nuclear receptors (RAR) $-\alpha-\beta-\gamma$
7. Transcription process
8. Translation
9. Degradation of RA (P 450)

**Table II.** Rate of Conversion of ($^3$H) ROL to ($^3$H)RA by Protein[a] Extracts

| | n | ($^3$H)RA 1[b,c] |
|---|---|---|
| Human skin | | |
| Normal | 6 | Traces |
| Psoriatic plaque | 6 | $0.33 \pm 0.07$ |
| Human keratinocytes[d] | | |
| Differentiating | 3 | 4.5 (14×) |
| Non-differentiated | 3 | Traces |

[a] For technical details see [10].
[b] 2 mM NAD.
[c] pmol/h/mg prot.
[d] From [10].

**Table III.** Hexenol and Retinol Dehydrogenase Activities

| | Hexenol (15 mM) nmol/h/mg | Retinol (0.6 $\mu$M) pmol/h/mg |
|---|---|---|
| Human skin | | |
| Normal | 3.7 | 0 |
| Psoriatic plaque | 3 | $0.33 \pm 0.07$ |
| Human keratinocytes | | |
| Differentiated | 1.3 | 4.5 |

**Table IV.** Rate of Conversion of ($^3$H)RAL to Either ($^3$H)ROL or ($^3$H)RA (In Vitro)

| | n | ($^3$H)ROL Formed[a] NADH[b] | ($^3$H)RA Formed[a] NAD[c] |
|---|---|---|---|
| Human skin | | | |
| Normal | 6 | $1 \pm 0.3$ | $1.5 \pm 0.5$ |
| Psoriatic Plaque | 6 | $2.5 \pm 0.2$ | $1.2 \pm 0.3$ |
| Human keratinocytes[d] | | | |
| Differentiating | 3 | 8.2 | 51.6 |
| Non-differentiated | 3 | 8.0 | 14.4 |

[a] pmol/h/mg prot.
[b] 5 mM.
[c] 2 mM.
[d] From [10].

These studies indicated that in keratinocytes the enzymatic conversion of retinol into retinoic acid, a process that might be a key regulatory event, is i) dependent on the stage of differentiation of the keratinocytes (interestingly, the cells that constitutively express the highest levels of CRABP also expressed the highest enzymatic activity; ii) very slow (its rate is in the order of pmol/h/mg protein) as compared to pig kidney cell lines [7] and rat tissues [8] (where it is in the range of nmol/h/mg protein); and iii) limited by the rate of formation of retinal and dependent on the NAD-NADH ratio in the tissue. The same techniques were then used for the study of cytosolic extracts of keratomed psoriatic plaques (keratome set at 280 $\mu$m). Table II shows that, as compared to normal skin (non-lesional psoriatic skin has not been studied so far), these extracts show measurable enzymatic activity that converts ($^3$H)retinol into RA. This is not related to alcohol deshydrogenase activity because, as shown in Table III, this activity is present in normal epidermis where retinol deshydrogenase activity is not detectable.

It therefore appears that in psoriatic plaques the rate of formation of RA from retinol is higher than in normal human epidermis. This correlates, as in differentiating cultured keratinocytes, with higher levels of CRABP. The link between these two observations is under study. It is also interesting in this context that at least in vitro, preliminary results show that synthetic retinoids such as RA, 13-cis-RA and acitretin inhibit the conversion of retinol into RA (manuscript in preparation).

When the transformation of ($^3$H)retinal by cytosolic extracts of psoriatic plaque was studied, it appeared that both normal epidermis and psoriatic plaques were able either to reduce retinal into retinol or to oxidize it into retinoic acid. The former reaction was higher in psoriatic plaques (Table IV). It appears that the ratio of the co-factors, NAD and NADH, is important in directing the reaction when retinal is used as substrate.

With the identification of retinoic acid nuclear receptors [12], the study of retinoic acid metabolism has become increasingly important. Indeed the amount of ligand that has access to the receptor might be one of the regulatory events. It is not beyond possibility that the enzymatic systems that transform retinol and retinal into retinoic acid play such a regulatory role within target cells. Preliminary experiments show that synthetic retinoids down-regulate the transformation of retinol into retinoic acid.

*We are grateful to Ms. R. Holz and E. Leeman for their skilled technical assistance.*

## REFERENCES


1. Siegenthaler G, Saurat JH: Therapy with a synthetic retinoid-(RO 10-1670) etretin-increase the cellular retinoic acid-binding protein in nonlesional psoriatic skin. J Invest Dermatol 87:122–124, 1986
2. Nervi C, Grippo JF, Sherman MI, Georg MD, Jetten AM: Identification and characterization of nuclear retinoic acid-binding activity in human myeloblastic leukemia HL-60 cells. Proc Natl Acad Sci USA 86:5854-5858, 1989
3. Siegenthaler G, Saurat JH, Ponec M: Terminal differentiation in cultured human keratinocytes is associated with increased levels of cellular retinoic acid-binding protein. Exp Cell Res 178:114-126, 1988
4. Hirschel-Scholz S, Siegenthaler G, Saurat JH: Ligand specific and non-specific in vivo modulation of human epidermal cellular retinoic acid-binding protein (CRABP). Eur J Clin Invest 19:220–227, 1989
5. De Leenheer AP, Lambert WE, Clarys I: All trans-retinoic acid: measurement of reference values in human serum by high performance liquid chromatography. J Lipid Res 23:1362-1367, 1982
6. Williams JB, Napoli JL: Metabolism of retinoic acid and retinol during differentiation of F9 embryonal carcinoma cells. Proc Natl Acad Sci USA 82:4658-4662, 1985
7. Napoli JL: Retinol metabolism in LLC-PK$_1$ cells. J Biol Chem 261:13592-13597, 1986
8. Napoli JL, Race KR: The biosynthesis of retinoic acid from retinol by rat tissues in vitro. Arch Biochem Biophys 255:95-101, 1987
9. Connor MJ: Terminal-group oxidation of retinol by mouse epidermis. Biochem J 244:489-492, 1987
10. Siegenthaler G, Saurat JH, Ponec M: Retinol and retinal metabolism. Relationship to the state of differentiation of cultured human keratinocytes. Biochem J 268:371–378, 1990
11. Siegenthaler G, Hotz R, Leemans E, Saurat JH: In vitro retinol and retinal metabolism in human skin (abstr). J Invest Dermatol 92:519, 1989
12. Krast A, Kastner P, Petkovich M, Zelent A, Chambon P: A third human retinoic acid receptor, h RAR $\gamma$. Proc Natl Acad Sci USA 86:5310-5314, 1989